

## MEM Elution Final Report

Test Article: Electrospun Cellulose  
 Purchase Order: P2186193  
 Laboratory Number: 595759  
 Study Received Date: 19 Aug 2011  
 Test Procedure(s): Standard Test Protocol (STP) Number: STP0032 Rev 07

**Summary:** The Minimal Essential Media (MEM) Elution test was designed to determine the cytotoxicity of extractable substances. An extract of the test article was added to cell monolayers and incubated. The cell monolayers were examined and scored based on the degree of cellular destruction. All test method acceptance criteria were met.

**Results:**  
 Test Article:


Results Pass/Fail	Scores				Extraction Ratio	Amount Tested / Extraction Solvent Amount	Post Extraction Appearance
	#1	#2	#3	Average			
Pass	0	0	0	0	0.2 g/mL	6.7 g / 33.5 mL	Clear


**Controls:**

Identification	Scores				Extraction Ratio	Amount Tested / Extraction Solvent Amount	Post Extraction Appearance
	#1	#2	#3	Average			
Negative Control - Polypropylene Pellets	0	0	0	0	0.2 g/mL	4 g / 20 mL	Clear
Media Control	0	0	0	0	N/A	20 mL	Clear
Positive Control - Latex Natural Rubber	4	4	4	4	0.2 g/mL	4 g / 20 mL	Clear

**Acceptance Criteria:** The United States Pharmacopeia & National Formulary (USP 87) states that the test article meets the requirements, or receives a passing score (**Pass**) if the reactivity grade is not greater than grade 2 or a mild reactivity. The ANSI/AAMI/ISO 10993-5 standard states that the achievement of a numerical grade greater than 2 is considered a cytotoxic effect, or a failing score (**Fail**).

Nelson Laboratories acceptance criteria was based upon the negative and media controls receiving "0" reactivity grades and positive controls receiving a 3-4 reactivity grades (moderate to severe). The test was considered valid as the control results were within acceptable parameters.

  
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 Technical Reviewer

  
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 Study Director

Christine Jensen



25 Aug 2011  
 Study Completion Date

The cell monolayers were examined microscopically. The wells were scored as to the degree of discernable morphological cytotoxicity on a relative scale of 0 to 4:

Conditions of All Cultures	Reactivity	Grade
No cell lysis, intracytoplasmic granules.	None	0
Not more than 20% rounding, occasional lysed cells.	Slight	1
Not more than 50% rounding, no extensive cell lysis.	Mild	2
Not more than 70% rounding and lysed cells.	Moderate	3
Nearly complete cell destruction.	Severe	4

The results from the three wells were averaged to give a final cytotoxicity score.

**Procedure:** The amount of test material extracted was based on ANSI/AAMI/ISO and USP surface area or weight recommendations. Test articles and controls were extracted in 1X Minimal Essential Media with 5% bovine serum for 24-25 hours at  $37 \pm 1^\circ\text{C}$  with agitation. Multiple well cell culture plates were seeded with a verified quantity of industry standard L-929 cells (ATCC CCL-1) and incubated until approximately 80% confluent. The test extracts were filtered to avoid bacterial contamination and added to the cell monolayers in triplicate. The cells were incubated at  $37 \pm 1^\circ\text{C}$  with  $5 \pm 1\%$   $\text{CO}_2$  for  $72 \pm 3$  hours.

## Agar Overlay Final Report

Test Article: Electrospun cellulose  
 Purchase Order: P2186193  
 Laboratory Number: 595758  
 Study Received Date: 19 Aug 2011  
 Test Procedure(s): Standard Test Protocol (STP) Number: STP0031 Rev 07

**Summary:** The Agar Overlay test was designed to determine the cytotoxicity of diffusible components from materials or solutions. A layer of agar was added over a cell monolayer to act as a cushion to protect the cells from mechanical damage while allowing the diffusion of leachable materials. The test articles were then placed on top of the agar layer and incubated. The cell monolayers were examined and scored based on the degree of cellular destruction. All test method acceptance criteria were met.

**Results:**

Identification	Amount Tested	Score #1	Score #2	Score #3	Average
Negative Control – Polypropylene Pellets	≥ 100 mm <sup>2</sup> per well	0	0	0	0
Positive Control – Latex Natural Rubber	≥ 100 mm <sup>2</sup> per well	4	4	4	4
Test Article	≥ 100 mg per well	0	0	0	0

**Acceptance Criteria:** The United States Pharmacopeia & National Formulary (USP 87) states that the test article meets the requirements if the reactivity grade is not greater than grade 2 or a mild reactivity. The ANSI/AAMI/ISO 10993-5 standard states that the achievement of a numerical grade greater than 2 is considered a cytotoxic effect. Nelson Laboratories acceptance criteria was based upon the negative control receiving "0" reactivity grades and positive control receiving 3-4 reactivity grades (moderate to severe).

**Procedure:** Six well cell culture plates were seeded with a verified quantity of industry standard L-929 cells (ATCC CCL-1) and incubated at 37 ± 1°C with 5 ± 1% CO<sub>2</sub> until approximately 80% confluent. The agar overlay consisted of an equal mixture of 2X agar (1.0%) and 2X MEM + 10% bovine calf serum. Solid test articles were placed directly on the solidified agar overlay testing ≥ 100 mm<sup>2</sup> per test well. Liquid or gel test articles were applied to sterile filter discs testing no less than 0.1 mL per well. Powders, resins, or irregular materials were placed directly onto the solidified agar, testing no less than 100 mg per well. Positive and negative reference controls were included with each assay.

  
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25 Aug 2011  
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All tests were performed using three test wells per test article. After the addition of the test articles, the cell culture plates were incubated as described above for 24-26 hours. Following incubation, cells were evaluated microscopically using the evaluation criteria outline below:

Grade	Description Of Zone
0	No detectable zone around or under the test article.
1	Some malformed or degenerate cells under the test article.
2	Zone limited to area under the test article.
3	Zone extends to 1.0 cm beyond the test article.
4	Zone greater than 1 cm in extension from test article.

The results from the three wells were averaged to give an average cytotoxicity score.